

Hepatitis C virus entry and glucocorticosteroids

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COMMENTARY ON:

Glucocorticosteroids increase cell entry by hepatitis C virus. Ciesek S, Steinmann E, Iken M, Ott M, Helfritz FA, Wappler I, Manns MP, Wedemeyer H, Pietschmann T. *Gastroenterology*. 2010;138:1875–84. Copyright (2010). Abstract reprinted with permission from Elsevier.

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Background & Aims: Corticosteroids are used as immunosuppressants in patients with autoimmune disorders and transplant recipients. However, these drugs worsen hepatitis C virus (HCV) recurrence after liver transplantation, suggesting that they may directly exacerbate HCV infection.

Methods: The influence of immunosuppressive drugs on HCV replication, assembly, and entry was assessed in Huh-7.5 cells and primary human hepatocytes using cell culture- and patient-derived HCV. Replication was quantified by immunofluorescence, luciferase assays, quantitative reverse-transcriptase polymerase chain reaction, or core enzyme-linked immunosorbent assays. Expression of HCV entry factors was evaluated by cell sorting and immunoblot analyses.

Results: Glucocorticosteroids slightly reduced HCV RNA replication but increased efficiency of HCV entry by up to 10-fold. This was independent of HCV genotype but specific to HCV because vesicular stomatitis virus glycoprotein-dependent infection was not affected by these drugs. The increase in HCV entry was accompanied by up-regulation of messenger RNA and protein levels of occludin and the scavenger receptor class B type I – two host cell proteins required for HCV infection; increase of entry by glucocorticosteroids was ablated by RU-486, an inhibitor of glucocorticosteroid signalling. Glucocorticosteroids increased propagation of cell culture-derived HCV approximately 5- to 10-fold in partially differentiated human hepatoma cells and increased infection of primary human hepatocytes by cell culture- and patient-derived HCV.

Conclusions: Glucocorticosteroids specifically increase HCV entry by up-regulating the cell entry factors occludin and scavenger receptor class B type I. Our data suggest that the potential effects of high-

dose glucocorticosteroids on HCV infection in vivo may be due to increased HCV dissemination.

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Hepatitis C-associated liver failure is the leading indication for liver transplantation (LT). Hepatitis C virus (HCV) re-infection of the liver graft occurs immediately after transplantation with a rapid increase in HCV RNA post-transplantation [1]. HCV recurrence is characterized by accelerated histological progression, with up to 30% of patients developing cirrhosis after five years following LT [1]. A prophylactic strategy for prevention of re-infection is lacking and interferon-based antiviral therapies have limited efficacy and tolerability in LT recipients [2]. Thus, recurrent liver disease with poor outcome has become an increasing problem facing hepatologists and transplant surgeons [2].

Viral and host factors contribute to HCV re-infection and progression of liver disease. Viral factors include efficiency of entry, replication, and production of progeny virus. Host factors include immune responses and graft- or donor-related factors. Advanced donor age and the use of immunosuppression have been postulated as risk factors for more rapid fibrosis following LT [3]. Furthermore, the administration of high doses of glucocorticosteroids (GCs) appears to be associated with the severity of HCV recurrence, increased mortality, and graft loss in LT recipients [3].

To better understand the impact of immunosuppressive drugs on HCV infection, an elegant study from Thomas Pietschmann's laboratory investigated the effect of GC, cyclosporin A, tacrolimus, mycophenolate sodium, basiliximab, evrolimus, and azathioprine on the HCV life cycle [4]. To identify virus-drug interactions, Ciesek et al. studied HCV entry, replication, and assembly in permissive Huh-7.5 hepatoma cells and primary human hepatocytes using HCV pseudoparticles and infectious virions. Using this approach, Ciesek et al. uncovered that GCs enhance HCV entry and dissemination into hepatocytes. Prednisolone (starting at 10–50 µg/ml) increased the efficiency of HCV entry by up to 10-fold whereas the other immunosuppressive compounds had no effect. The GC effect was independent of HCV genotype but specific to HCV because vesicular stomatitis virus glycoprotein-dependent infection was not affected by these drugs. Although GC slightly affected HCV RNA replication, the pronounced stimulation of HCV entry was dominant thus increasing propagation of cell culture-derived HCV approximately 5- to 10-fold in partially differentiated human hepatoma

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Abbreviations: CLDN1, claudin-1; HCV, hepatitis C virus; HCVcc, cell culture-derived HCV; HCVpp, HCV pseudoparticles; LDLR, low density lipoprotein receptor; OCLN, occludin; SR-BI, scavenger receptor class B type I.



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cells and increased HCV infection of primary human hepatocytes [4]. Using kinetic studies of HCV infection, the authors provide evidence that GCs accelerate the speed of HCV cell entry. Finally, by using different densities of Huh7.5 cells infected with recombinant reporter viruses in the presence of prednisolone, the authors demonstrate that GC enhanced HCV dissemination in a cell-density-dependent manner [4].

HCV entry into hepatocytes is a complex and multistep process requiring several entry factors, including scavenger receptor class B type I, CD81 and tight junction proteins claudin-1 (CLDN1), and occludin (OCLN) (for review see [5,6]). To further investigate the mechanisms mediating GC-induced enhanced viral entry, Ciesek et al. analyzed the expression of HCV cell entry factors. While expression of CD81, CLDN1 was not affected by GCs, expression of SR-B1 and OCLN was increased in a dose-dependent manner. The effects of glucocorticoids are mediated by the glucocorticoid receptor [7]. The glucocorticoid receptor binds to specific DNA

sequences – glucocorticoid-response elements – in the 5′-flanking region of target genes and transactivates gene transcription [7]. Indeed, a glucocorticoid-response element was recently identified within the *OCLN* gene [8]. Thus, transactivation of *OCLN* gene expression by GCs [8] may be responsible for the enhanced entry factor expression and viral entry (Fig. 1). In line with this interpretation, Ciesek et al. report that ablation of GC signalling by RU-486 prevents up-regulation of OCLN and SR-B1 and in turn also enhancement of HCV cell entry.

What are the clinical implications of these findings? Viral cell entry and dissemination are the very first steps of HCV infection and required for maintenance of chronic infection in the patient. Furthermore, enhanced viral entry and escape from neutralizing antibodies are key determinants of re-infection of the liver graft during liver transplantation [9]. The findings of Ciesek et al. suggest that the potential effects of high-dose GCs on viral load *in vivo* may be due to increased HCV entry and dissemination.

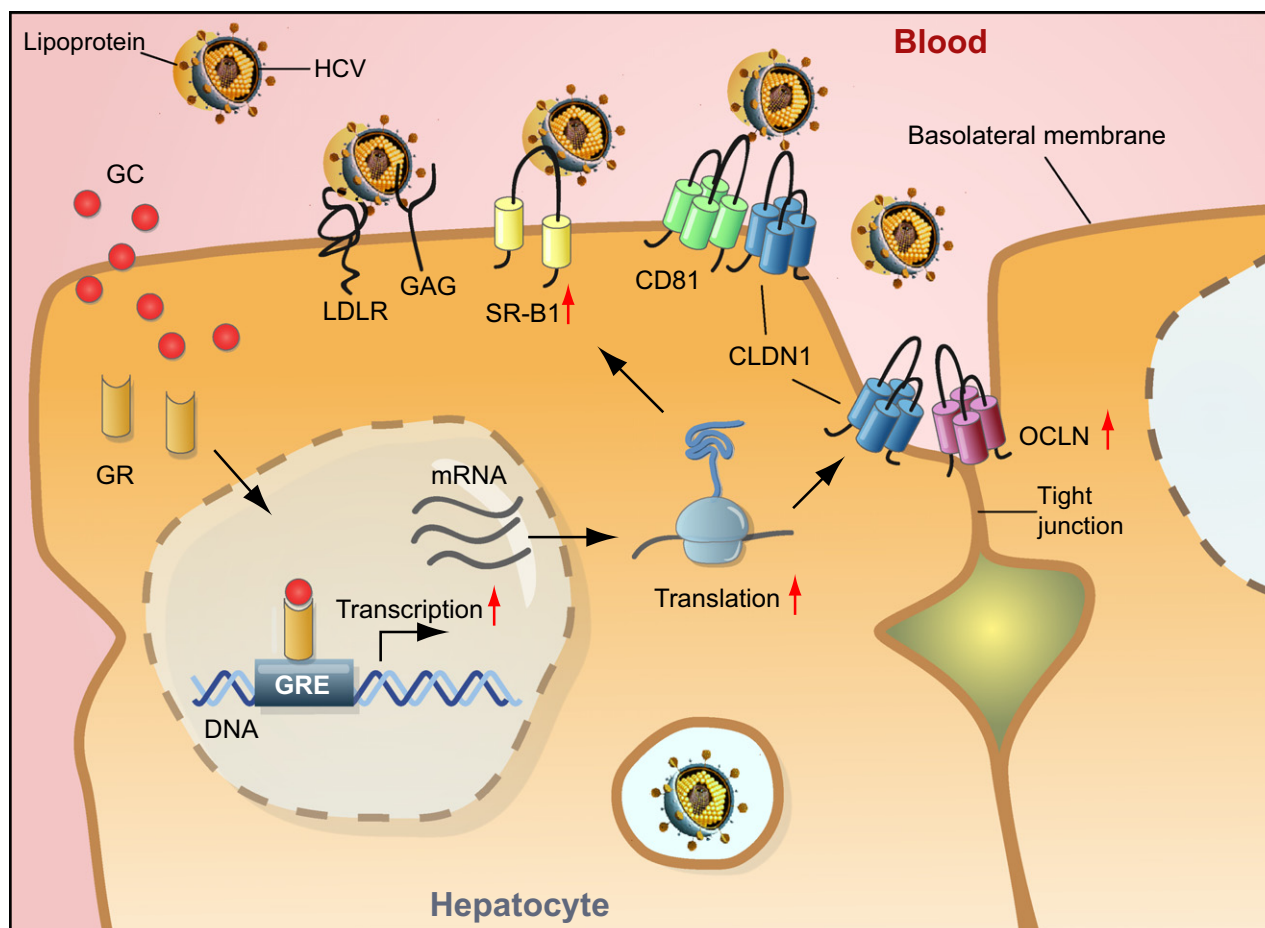


Fig. 1. Model of glucocorticoid-induced enhanced HCV entry into hepatocytes. HCV entry into hepatocytes is a multistep process: glycosaminoglycans (GAG) and low-density lipoprotein receptor (LDL-R) represent putative attachment sites before the virus interacts with cell entry factors including scavenger receptor class B type I (SR-BI), CD81, claudin-1 (CLDN1), and occludin (OCLN). For CLDN1 both junctional and non-junctional forms have been described (for review see Zeisel et al. [6]). Glucocorticosteroids (GCs) are believed to enhance HCV entry by the following events [3]: In the cytoplasm, GCs bind to the glucocorticoid receptor (GR). In the nucleus, the complex binds to specific DNA sequences – the glucocorticoid-response element (GRE) – and transactivates gene transcription with subsequent overexpression of response genes including HCV cell entry factor occludin [8]. GCs also enhance SR-B1 gene expression by a yet unknown mechanism [3]. Overexpression of occludin and SR-B1 facilitates HCV entry into hepatocytes and viral dissemination [3]. Abbreviations: GC – glucocorticosteroid, GAG – glycosaminoglycans, GR – glucocorticoid-receptor, GRE – glucocorticoid-response element, HCV – hepatitis C virus, SR-BI – scavenger receptor class B type, CLDN1 – claudin 1, LDLR – low-density lipoprotein receptor; OCLN – occludin.

International Hepatology

Based on their dose-dependent observations on GC-HCV interactions obtained in cell culture models, the authors conclude that steroid bolus treatment (>250 mg prednisolone/day) of HCV-infected individuals, which results in plasma levels up to 50 µg/ml, may foster virus dissemination through facilitation of virus entry into hepatocytes, thus aggravating HCV recurrence. Indeed, several studies demonstrated that high-dose steroid therapy leads to a sudden and dramatic increase in viremia levels in the early post-operative period [8]. Furthermore, a meta-analysis suggests that immunosuppression regimens without steroids are associated with a reduction in the severity of the recurrence of hepatitis after LT [10].

Although further studies are needed to better understand the mechanism of accelerated progression of HCV-induced liver disease in transplant patients, the findings of Ciesek et al. have uncovered a novel mechanism in HCV-drug interactions playing a role in this process. Enhanced HCV entry and dissemination induced by high-dose GCs may not only have an important clinical relevance for the understanding of the pathogenesis of HCV infection but could provide a rationale for the development of improved immunosuppressive therapies for the HCV-infected patient.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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